BRIC 21:
Global transcriptome profiling to identify cellular stress mechanisms responsible for spaceflight-induced antibiotic resistance

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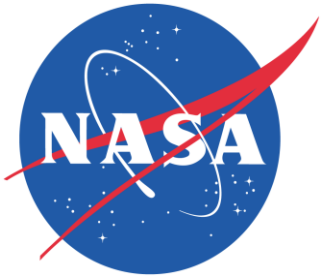

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OVERVIEW

- Hypothesis
- Investigation
- Goals and objectives
- Measurement approach
- Importance and Reason for ISS
- Expected results
- How Results will advance the field
- Earth benefits/spin-off applications

CENTRAL HYPOTHESIS:

- **Exposure to the human spaceflight environment causes stresses (i.e. “spaceflight syndrome”) which can lead to increased antibiotic resistance in bacterial opportunistic pathogens.**

INVESTIGATION

- **Two harmless surrogates of opportunistic pathogens (*Bacillus subtilis* and *Staphylococcus epidermidis*) will be cultured in microgravity on ISS for 24 +/- 4 hours, frozen, and returned to Earth.**
- **Global stress responses will be measured in comparison to ground controls using Whole Transcriptome Shotgun Sequencing (a.k.a. "RNA-Seq").**
- **The spectrum of antibiotic resistance from flight samples and ground controls will be compared using the Omnilog phenotype microarray system containing a large collection of antibiotics.**
- **The rate of spontaneous mutation to resistance to the antibiotic Rifampicin (RFM) will be measured and compared to ground controls.**

GOALS AND OBJECTIVES

- **Characterize changes in the transcriptomes of bacteria exposed to human spaceflight stresses, esp. microgravity, focusing on global stress responses.**
- **Compare the extent and levels of resistance to various antibiotics resulting from spaceflight compared to ground controls.**
- **Compare the mutation rates of bacteria grown in space vs. ground controls.**

MEASUREMENT APPROACH

- **Total RNA will be isolated from flight and ground-control cultures and their global transcriptomes compared using RNA-Seq. Genes significantly up- or down-regulated will be identified and further investigated for their stress relevance.**
- **Upon return, cultures will be inoculated immediately into 96-well Omnilog antibiotic plates (PM-11C, 12B, 13B) and assayed for the level of resistance to a battery of antibiotics, relative to ground control cultures.**
- **Cells returned from ISS will be plated for (i) total viable cells and (ii) RFM-resistant mutants. Mutation frequencies will be computed and compared relative to ground controls. Mutations will be identified by nucleotide sequencing.**

IMPORTANCE AND REASON FOR ISS

- **Prior evidence that astronaut immune function deteriorates during prolonged spaceflight.**
- **Prior evidence suggesting virulence and antibiotic resistance of some (but not all) bacteria increases during spaceflight.**
- **Thus, development of multiple antibiotic resistance in opportunistic pathogens is of importance to astronaut health.**
- **Prior evidence that the bacterial "spaceflight syndrome" appears to be organism-specific, and no fundamental underlying mechanism has yet emerged.**

EXPECTED RESULTS

- **<u>If the Central Hypothesis is supported:</u>**
  - **Transcription of global stress response genes will be up-regulated significantly in ISS-grown samples compared to ground control samples.**
  - **ISS-grown cells will exhibit resistance to a greater number of antibiotics, and/or to higher concentrations, than ground-control cells.**
  - **Mutation frequencies to RFM resistance will be significantly higher in the ISS-grown cultures than the ground control cultures.**

HOW RESULTS WILL ADVANCE THE FIELD

- **Little is currently known at the molecular level about the global cellular stress responses of microbes in the human space flight environment and how they impact pathogenicity and antibiotic resistance.**
- **This project will enhance our knowledge of how potentially harmful microbes respond and adapt to the spaceflight environment.**
- **Knowledge gained from the project will ultimately benefit astronaut health during long-term missions.**

EARTH BENEFITS / SPIN-OFF APPLICATIONS

- **Development and dispersal of multiply-resistant pathogenic bacteria is a chronic problem in clinical settings.**
- **Clinical settings in some ways mirror the human spaceflight environment; several people with compromised immune systems are housed in confined quarters for extended periods of time.**
- **Understanding how antibiotic resistance develops in space could lead to better methods for combating hospital-acquired infections.**